UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 27, 2010

MEMORANDUM

Subject: Efficacy Review for Ecaflo Anolyte;
EPA Reg. No. 82341-1; DP Barcode: D371839

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Applicant: Integrated Environmental Technologies, LTD
4235 Commerce Street
Little River, SC 29566

Formulation from the Label:

Active Ingredient(s)	% by wt.
Hypochlorous Acid	0.046%
Other Ingredients	99.954%
Total	100.000%

I. BACKGROUND

The product, EcaFlo® Anolyte, is an Agency-registered (Reg. No. 82341-1) hospital/medical use and general disinfectant (bactericide). The applicant is submitting data to support virucidal claims against Swine Influenza virus H1N1. Testing was conducted by Microbiotest, Inc. located at 105B Carpenter Drive in Sterling Virginia.

The data package contained a letter from the applicant's representative to the Agency (dated October 13, 2009), the proposed label (dated October 20, 2009), and one study (MRID 478931-01) with Statements of No Data Confidentiality and Good Laboratory Practice.

II. USE DIRECTIONS

The product is designed to be used as a disinfectant on hard, non-porous surfaces such as door handles, clean-up carts, light switches, sinks, tubs, tiles, toilets shower doors, floors, dressing or linen carts, hampers, diaper pails, toilet seats, bed pans, plastic mattress covers, and lockers. Directions on the proposed label provided the following information regarding the use of the product as a disinfectant: Apply EcaFlo Anolyte at 500 ppm FAC to hard, non-porous surfaces with a cloth, mop, or sponge. Treated surfaces must remain wet for 10 minutes. Allow surfaces to air dry. Food contact surfaces such as counters and tables must be rinsed with potable water.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides

The effectiveness of virucides must be tested using virological techniques that simulate the conditions under which the product is intended for use. For products with intended use on dry, inanimate environmental surfaces, carrier tests that are variations of either the AOAC Use-Dilution Method (for liquid surface disinfectants) or the AOAC Germicidal Spray Products Test (for surface spray disinfectants) must be used to produce virucidal data. The virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface (petri dish, glass slide, steel cylinder, etc.) for a specified exposure period at room temperature. The virus must be assayed by an appropriate virological technique testing a minimum of four determinations for each dilution. The protocol for the viral assay must include viral recovery, cytotoxicity controls, and ID-50 values. Test results should be reported as the reduction of the virus titer by the activity of the germicide (ID-50 of the virus control less the ID-50 of the test system) expressed as $\log_{10}$ and calculated by a statistical method. For virucidal data to be acceptable, the product must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. The calculated viral titers must be reported with the test results. Separate studies on two batches of product are required for each virus.

Supplemental Recommendations

Antimicrobial agents which claim to be "one-step" cleaner-disinfectants, or cleaner-sanitizers, or agents to be used in the presence of organic soil, must undergo appropriate efficacy testing modified to include a representative organic soil of 5% blood serum. A suggested method to simulate antimicrobial treatment of dry inanimate surfaces is to add the blood serum 5% v/v (19mL bacterial inoculum with 1mL blood serum) to bacterial inoculum prior to carrier contamination and drying. Control data should be produced as described in Supplemental Recommendation 6 of DIS/TSS-2 to confirm the validity of this test with this modification. The suggested organic soil level is appropriate for simulation of lightly to moderately soiled surfaces. For highly soiled surfaces, a prior cleaning step should be recommended on the product label. A suggested procedure for incorporating organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC Fungicidal Test involves adding 5% v/v blood serum directly to the test solution (e.g., 4.75 ml test solution + 0.25 ml blood serum) before adding 0.5 ml of the required level (5 X 10^6 /ml) of conidia.

IV. SUMMARY OF SUBMITTED STUDY

MRID 478931-01 "Virucidal Efficacy Test Using Swine Influenza Virus (H1N1)" against Ecaflo® Anolyte by S. Steve Zhou. Study conducted by Microbiotest, Inc., Laboratory Project Number 657-103. Study completed September 17, 2009.

This test was conducted against Swine Influenza Virus (H1N1), strain A/Swine/1976/31 (ATCC VR-99) using MDCK cells (ATCC CCL-34) as the host system. Two lots (Lot Nos. 812-01 and 812-02) of the product, Ecaflo Anolyte, were tested following Microbiotest protocol 657.1.08.14.09. The test agent was prepared by adding 2.42 mL of 300 ppm + 2.9% AOAC hard water to 10 mL of test concentrate (621 ppm) to obtain a final active concentration of 500 ppm. The stock virus culture contained 5% serum. Films of virus were prepared by spreading 0.4 mL of virus inoculum over 2 x 2 inch areas of glass carriers. The virus films were dried at ambient temperature for 30 minutes. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution for 10 minutes at 21°C. Following exposure, the plates were neutralized with 2.0 mL of Minimum Essential Medium supplemented with 1% Fetal Bovine Serum and 0.5% $Na_2S_2O_3$. The plates were scraped with a cell scraper to re-suspend the contents. Ten-fold serial dilutions were prepared, using Minimal Essential Medium supplemented with 1 µg/mL Trypsin. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with selected dilutions. The cultures were incubated for 4-6 days at 36±2°C in 5±1% CO_2. The cultures were re-fed, as necessary. Following incubation, the host cells were examined microscopically for the presence of infectious virions. Controls included those for cell viability/sterility, virus stock titer, plate recovery count, neutralizer effectiveness/viral interference, and cytotoxicity. The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the method of Spearman Karber.

V. RESULTS

MRID Number	Organism	Results			Dried Virus Count ($TCID_{50}$/ carrier)
			Lot 812-01	Lot 812-02	
478931-01	Swine Influenza Virus H1N1, A/Swine/ 1976/31 (ATCC VR-99)	10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.85}$
		$TCID_{50}$/ Carrier	$\leq 10^{1.5}$	$\leq 10^{1.5}$	

VI. CONCLUSIONS

1. The submitted data (MRID 478931-01) support the use of the product, Ecaflo Anolyte, as a hard surface disinfectant with virucidal activity against Swine Influenza Virus H1N1, A/Swine/ 1976/31 (ATCC VR-99) at a 500 ppm active concentration in the presence of light organic soil at room temperature with a contact time of 10 minutes. Complete inactivation was demonstrated at all dilutions. Controls were acceptable for valid tests. Viral titers were at least 10^4.

VII. RECOMMENDATIONS

1. The proposed label claims that the product, Ecaflo Anolyte, is an effective one-step disinfectant with virucidal activity against **Swine Influenza virus H1N1 ATCC VR-99** in the presence of moderate organic soil (5% serum) at a 500 ppm free available chlorine concentration with a contact time of 10 minutes on hard, nonporous surfaces. These claims are **acceptable** as they are supported by the submitted data.